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THE ULTRASTRUCTURE OF THE AFLAGELLATE SPERMATOZOON OF THE FRESHWATER TURBELLARIAN *HYDROLIMAX GRISEA* (PLATYHELMINTHES: PLAGIOSTOMIDAE)[1,2]

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The spermatozoön of *Hydrolimax grisea* was described by Hyman (1938; p. 16) as ". . . of the type characteristic of the Plagiostomidae, with a central long filamentous nucleus and winglike lateral protoplasmic expansions." An illustration and description of staining characteristics of spermatozoa in histological preparations of *H. grisea* were presented by Newton (1970). Spermatozoa of other plagiostomids have been figured or described by several authors in taxonomic studies (Bresslau, 1933; Hyman, 1938; Kepner, Stirewalt and Ferguson, 1941; Stirewalt, Ferguson and Kepner, 1942; Westblad, 1956; Karling, 1962; Kulinich, 1970, 1973). Plagiostomid spermatozoa are included in phylogenetic schema which have resulted from efforts at comparative spermatology (Hendelberg, 1969; 1970; *cf.* Bedini and Papi, 1970). Christensen (1961) provided the only other account of the ultrastructure of the spermatozoön of a plagiostomid turbellarian: the marine form *Plagiostomum morgani,* from Woods Hole, Massachusetts. Unfortunately, Christensen's brief abstract was not accompanied by electron micrographs.

Living mature spermatozoa of some marine plagiostomid species have been described by Jensen (1883), Böhmig (1891) and von Graff (1908, 1911). Only one of these authors, Jensen, observed movement by a spermatozoön.

Absence of flagella in the spermatozoa of plagiostomid turbellarians (Christensen, 1961; Hendelberg, 1969) requires another structural basis for sperm motility. The presence of "fibrils or tubules" immediately beneath the plasma membrane of the spermatozoön of *P. morgani* led Christensen (1961) to suggest that the region containing the tubules (*i.e.*, "the pellicle") is the basis for sperm motility in *Plagiostomum.* Cortical singlet microtubules in the aflagellate spermatozoön of *Hydrolimax grisea* are reported in this communication. Additional ultrastructural details, of sperm nucleus and of cytoplasmic specializations, suggest a unique morphology for the unusual male gamete of *Hydrolimax.*

Materials and Methods

For bright-field microscopy, specimens of *Hydrolimax* were prepared according to the procedures of Newton (1970). Wet mount preparations of live

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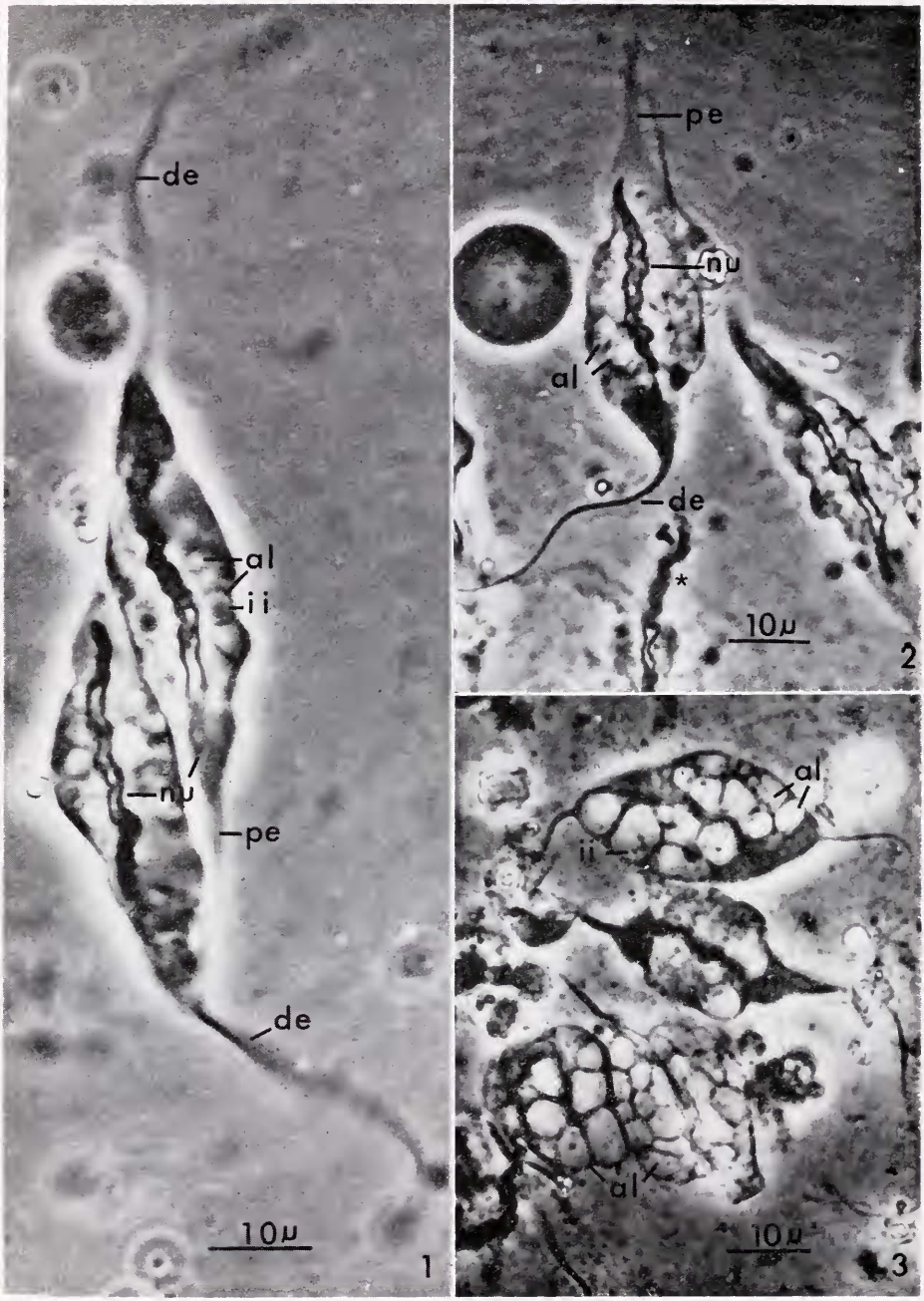

FIGURES 1–3. Phase contrast micrographs of living mature spermatozoa of *Hydrolimax grisea* released into pond water.

spermatozoa were studied and photographed with a Zeiss Photomicroscope I, using phase optics.

Two methods of fixation for electron microscopy were used: the procedure of Anderson and Personne (1970) for freshwater material; and a procedure of D. P. Costello (personal communication; described in Newton, 1974). The material was dehydrated in a graded series of ethanol, passed through propylene oxide and embedded in Epon-Araldite. Sections were cut on a Sorvall Porter-Blum MT-2 ultramicrotome with glass or diamond knives and stained with 3% uranyl acetate alone or in combination with 0.5% lead citrate. Maceration and negative staining of spermatozoa were done in 1% aqueous solution of phosphotungstic acid according to the method of Henley (1970). Both sectioned and negatively stained preparations were studied with a Zeiss 9S2 or an Hitachi HU-11B electron microscope.

Observations and Results

Light microscopy

The fixed, sectioned and stained male gamete of *Hydrolimax* has a central core of Feulgen-positive chromatin. This nucleus is fuchsinophilic and the cytoplasm stains light blue after Mallory's triple stain. In Giemsa smear preparations and with iron hematoxylin sperm cytoplasm is unstained but marked by a distinct boundary. All these observations were reported previously (Newton, 1970). One end of the nucleus is rounded in contour, whereas the other end terminates in a sharp point.

When observed with phase optics, however, the living mature spermatozoön of *H. grisea* is considerably more complex in structure (Figs. 1–3) than is shown in the usual histological preparations. The size of the spermatozoön varies from 80 to 100 μ in length and from 9 to 13 μ in width. The cell body is fusiform but irregular in contour. The vermiform nucleus extends almost the full length of the gamete and is surrounded by alveolate cytoplasm. Within the alveoli of the sperm cytoplasm are spherical or irregular inclusions (Figs. 1 and 3) which, in living spermatozoa, exhibit Brownian movement.

No flagella were seen in the spermatozoön of *Hydrolimax*. Movement by the spermatozoön was not observed. What is "head" and "tail" of the gamete remains unknown. For purposes of description, the ends of the spermatozoön are here designated proximal and distal with respect to a residual body to which developing spermatozoa are attached (*cf.* Jensen, 1883; Böhmig, 1891). The proximal end of the spermatozoön is circular in optical transsection and is translucent. The

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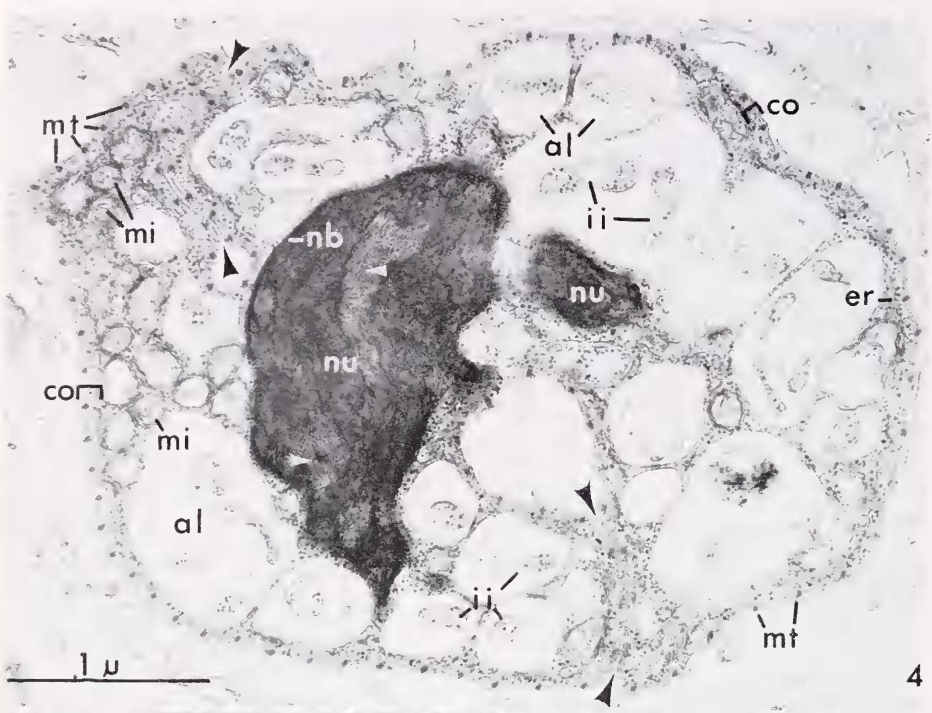
Figure 1. Spermatozoa with spherical inclusions in alveoli. Distal ends extend out of plane of focus. The following abbreviations represent: al, alveolus; de, distal end; ii, intra-alveolar inclusion; nu, sperm nucleus; and pe, proximal end.

Figure 2. Distal end of spermatozoön is in "side" view. Compare with proximal end. Asterisk marks a nucleus of a cytolyzed spermatozoön; note attached debris. Abbreviations are as in Figure 1.

Figure 3. Alveoli, and irregular inclusions, become more distinct when sperm are compressed by weight of overlying coverslip. One spermatozoön (center) has cytolyzed. Abbreviations are as in Figure 1.



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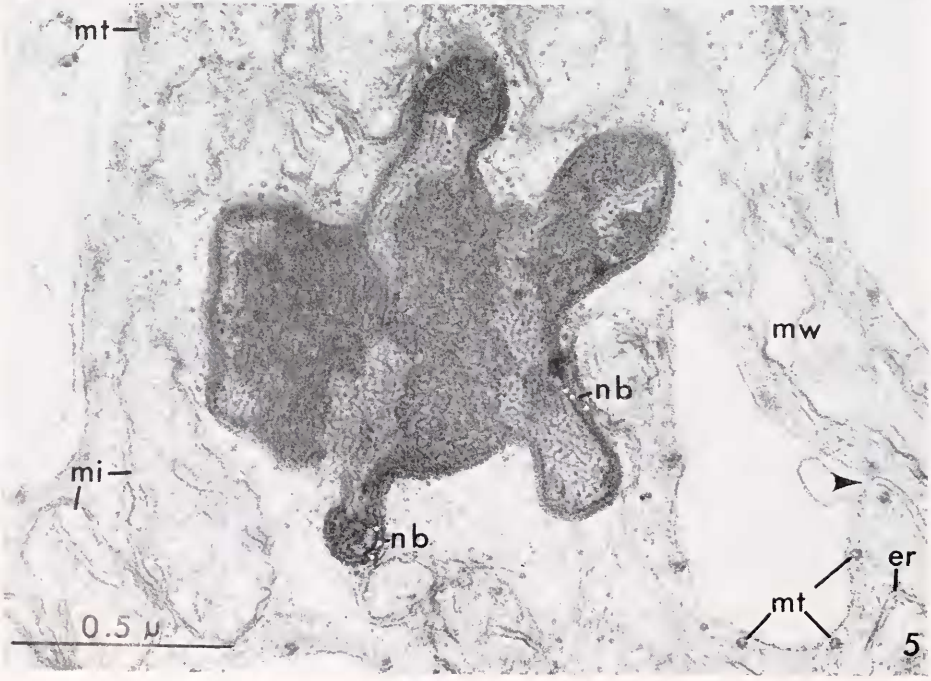

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elongate distal end is flattened, as can be verified by careful examination of the spermatozoön through many planes of focus, and is often bent in the plane perpendicular to the plane of flattening.

Electron microscopy

In the parenchyma of adult turbellarians which have recently copulated, spermatozoa aggregate in the vicinity of oöcytes outside the oviducts (Newton, 1970; Fig. 17; *cf.* Christensen, 1961). From sections of such aggregations, spermatozoan ultrastructure can best be studied, for these male gametes are undoubtedly mature: the spermatozoa have migrated through the parenchyma to fertilize the eggs.

In cross-section, the spermatozoön of *Hydrolimax* has an irregular boundary (Fig. 4), the outline of which varies among spermatozoa and among different sections of the same spermatozoön. Three major ultrastructural features may be distinguished in sections of the gamete: (1) the cortex, with its many microtubules; (2) the alveolate cytoplasm; and (3) the nucleus.

The cortex of the spermatozoön of *Hydrolimax* includes cytoplasm immediately beneath the cell surface containing the microtubules (Figs. 4 and 6). The inner boundary of the cortex is usually marked by a double membranous structure, apparently smooth endoplasmic reticulum. Other organelles, including mitochondria and alveoli, are found beneath the cortical cytoplasm. The cortex is of medium electron density, granular and varies from 430 Å to over 1000 Å in thickness. An indication of special physical properties of cortical cytoplasm is the absence of organelles, other than microtubules, from the cortex. Often the cortex is folded in toward the nucleus (Figs. 4 and 5), especially in the proximal half of the cell. In longitudinal sections of spermatozoa the cortex is often in close proximity to the nucleus for relatively long distances. Infolding of the sperm cortex may serve to divide the cytoplasm of the spermatozoön into lateral cytoplasmic extensions (*cf.* Hyman, 1938). In the spermatozoan mid-region, the cortex does not fold in toward the nucleus.

Figures 7 and 8 are micrographs of successively distal sections of spermatozoa. The distal terminus of the sperm nucleus is seen in section in Figure 7, along with some membranous elements, here difficult to identify. The cortex is thrown into several folds toward the nucleus. A transverse section through the flattened distal end of the spermatozoön (Fig. 8) shows an endoplasm devoid of organelles, except for a suggestion of endoplasmic reticulum.

Within the cortex singlet microtubules spiral along the length of the spermatozoön (Figs. 4, 5, and 6). In any transverse section of a spermatozoön, the cortical singlet microtubules exhibit varying orientations with respect to the plane

FIGURE 4. Electron micrograph of transverse section of proximal end of spermatozoön. The cortex folds in toward the nucleus in two places (large arrows). Small white arrows point out fibrous component of nucleus. Approximately 98 microtubular profiles can be counted in the cortex. Costello's fixative is used; uranyl acetate is used; co represents cortex; er, endoplasmic reticulum; mi, mitochondria; mt, microtubule; nb, nuclear boundary; other abbreviations as in Figures 1–3.

FIGURE 5. Transverse section of sperm nucleus (*cf.* Fig. 4) showing dark fibers (small arrows) in cross section. Large arrow points to infolding of cortex. Costello's fixative is used; uranyl acetate is used; mw represents membranous whorl; other abbreviations as in Figures 1–4.

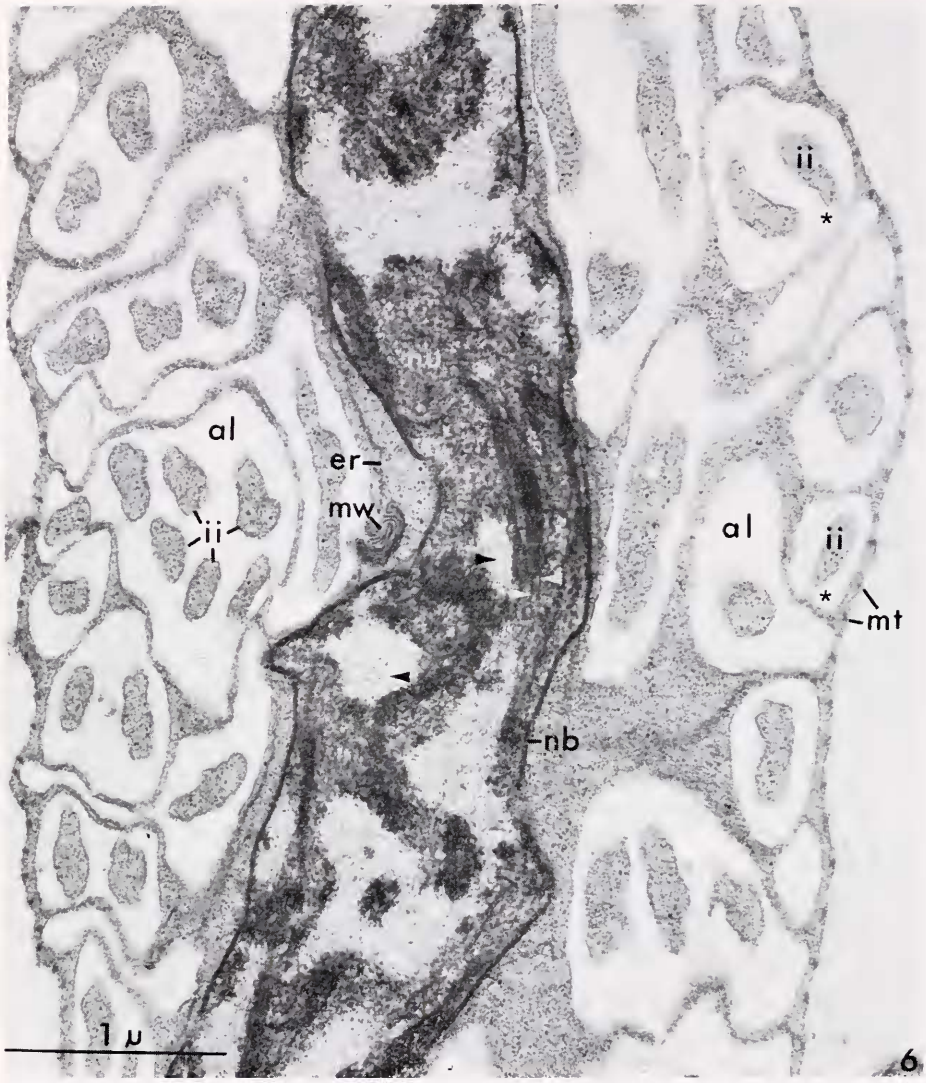


FIGURE 6. Longitudinal section of a spermatid mid-region. Arrows point to dark fibers of nucleus. Asterisks indicate connections between intra-alveolar particles and sperm cytoplasm. Anderson-Personne fixative is used; uranyl acetate, and lead citrate are used; abbreviations are as in Figures 1–5.

of section, from perpendicular to oblique, to parallel (Figs. 4 and 5). The longitudinal section in Figure 6 shows microtubules in transverse section. The number of cortical singlet microtubules in cross-sections of spermatozoan mid-regions ranges from 90 to 116. The singlets are widely separated in the sperm mid-region, *ca.* 1200 to 1800 Å, and closely spaced in the distal end, *ca.* 100 to 200 Å (*cf.* Figs. 4 and 8). There is no evidence of bridging between microtubules. No connection between

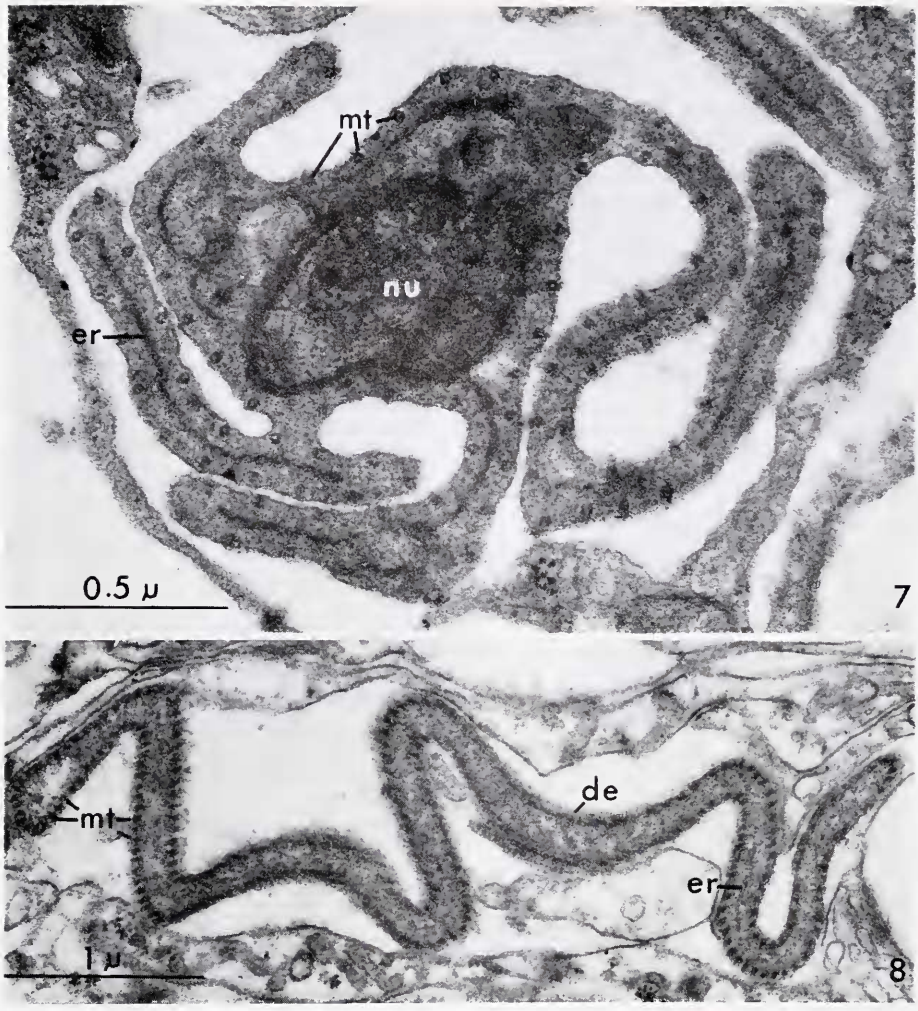


FIGURE 7. Distal nuclear terminus is surrounded by sparse cytoplasm with some membranous elements. Cortex is highly folded. Costello's fixative is used; uranyl acetate is used; abbreviations are as in Figures 1–5.

FIGURE 8. Transverse section of flattened distal end. Note numerous microtubules. Costello's fixative is used; uranyl acetate is used; abbreviations are as in Figures 1–5.

the singlets and the sperm cell surface is seen. An accurate count of microtubules is difficult due to vagaries in the shape of the spermatozoön and to oblique orientation of microtubules in the plane of section. The outside diameter of microtubules, calculated from measurements from transverse sections, is *ca.* 275 to 320 Å.

Maceration and negative staining of spermatozoa (from dissected seminal vesicles) provided excellent material for examination of cortical microtubules in the distal region of the spermatozoön (Figs. 9–12). Remnants of the sperma-

FIGURE 9. Cortical singlet microtubules revealed by maceration and negative staining with PTA. Numbers refer to microtubules in transects. Some of the microtubules are relatively straight whereas others noticeably bend into a wave pattern.

tozoan nucleus and the alveolate cytoplasm of the mid-region were extremely electron-dense after treatment with PTA. A few well macerated and spread distal ends of spermatozoa permitted a count of up to 114 microtubules. The microtubules terminate abruptly and decrease in number rapidly toward the end of the spermatozoön (Fig. 9).

Figure 10 demonstrates different orientations of microtubules within different regions of the same sperm cell. Apparently the distal end of the spermatozoöon was reflected back alongside the mid-region during maceration with PTA. The cortical singlets within the distal end are straight, at least more so than within the spermatozoan mid-region. It is interesting that a microtubule is straight in the distal end and spirally arrayed in the cortex of the mid-region of the spermatozoön. PTA digests of the proximal end likewise show straight microtubular arrays. The cortical singlet microtubules "change" their orientation from spiral to straight as they extend proximally or distally from the mid-region.

The spermatozoan microtubules in *Hydrolyimax* are *not* rigid structures: the cortical singlets bend. In Figure 10, some microtubules of the distal end are straight, whereas others have an irregular wave-like pattern. A similar microtubular arrangement of straight and "wavy" cortical singlets can be seen at lower magnification in Figure 9. In Figure 11 the radii of the microtubular arc shown at the arrows are approximately 0.46 μ—the smallest observed arc radius into which microtubules were bent. Bending through an arc radius of less than *ca.* 0.46 μ may produce breaks, likewise evident in Figure 11. Microtubule bending may have been caused by action of PTA or created during removal of macerating fluid.

The diameter of singlet microtubules in PTA preparations is *ca.* 270 to 300 Å The cortical singlets have a substructure suggested by alternately light and dark bands pitched relative to the microtubule axis. The electron-lucent bands, bounded by electron-opaque deposits of phosphotungstate, are the only subunits observed in the present material (Fig. 12; *cf.* Thomas and Henley, 1971). These subunits have a center-to-center spacing of 79 to 86 Å and pitch angles of 10° to 22°. No protofilaments were observed (see, however, Fig. 12). A dark line, *ca.* 34 to 40 Å wide, extends down the center of the singlets (Figs. 11 and 12). This striking linear feature of the microtubules may represent the "lumen" filled with electron-opaque phosphotungstate. It is possible that some substance within the singlet has a special affinity for PTA. The dark line appears to divide the subunits, creating the illusion of a "herring-bone" or double-element array of substructure. The subunits are not divided, however, but apparently are spirally arrayed round the central element of the microtubule, the "lumen."

The cortical singlets are apparently quite long; lengths up to 70 μ can be estimated from Figure 9 (the estimates include *only* the portions of the microtubules in the figure). Examination of a series of micrographs in a montage suggests microtubule lengths up to 100 μ or more. Single microtubules can extend from one end of the spermatozoön to the other.

The cytoplasmic alveoli of the spermatozoön are membrane-bound vesicles (Figs. 4, 5 and 6), in which are suspended membrane-bound inclusions of coarsely granular material. The alveoli also contain irregularly shaped particles of medium electron density. The significance of the alveoli and of their contents is unknown. Current studies of spermiogenesis at the EM level indicate that the alveoli originate

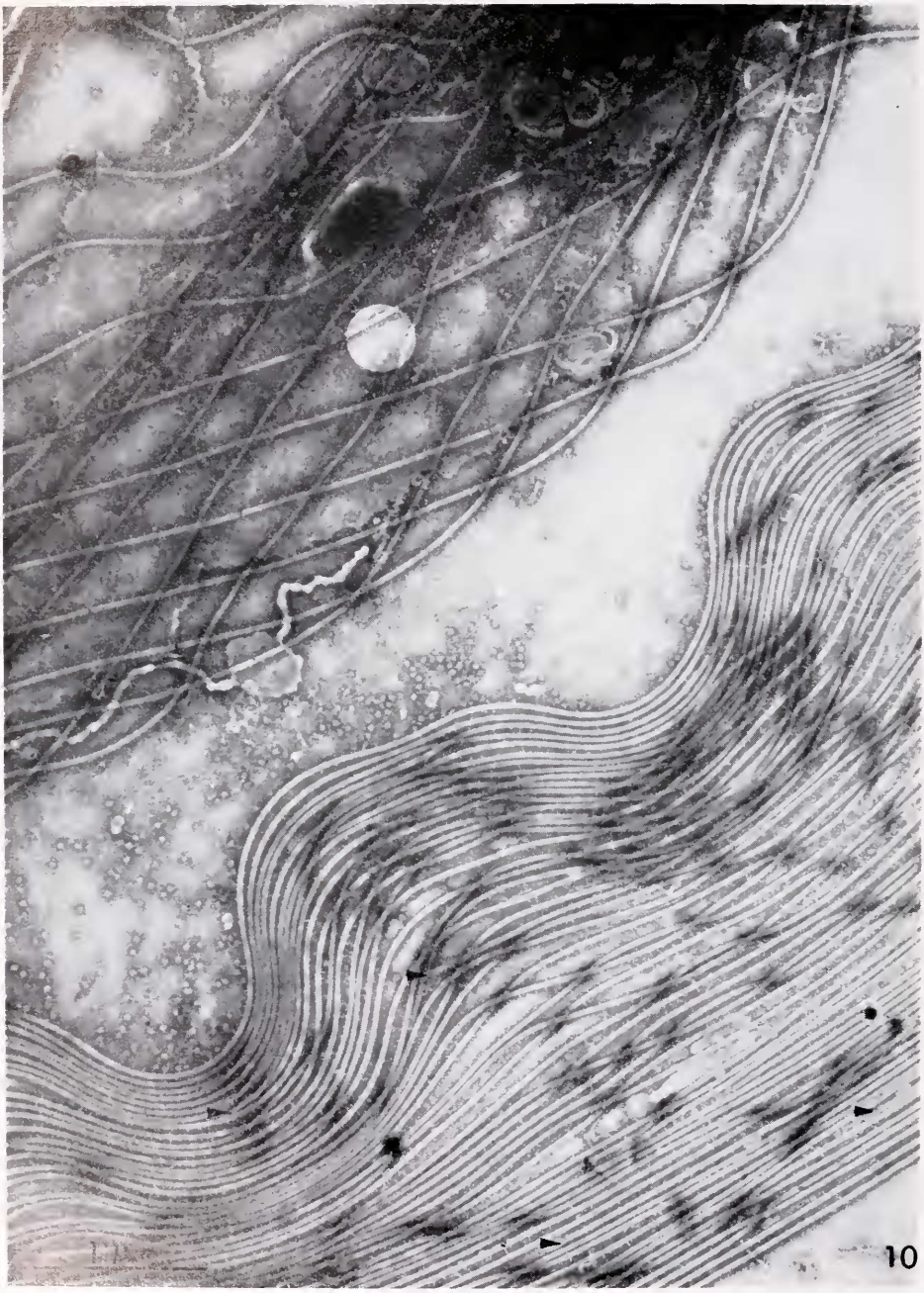


FIGURE 10 Cortical singlet microtubules of the mid-region of the spermatozoön have settled on the Formvar-carbon-coated grid in an apparent geometric projection of their spiral course along the sperm-cell body (top). In contrast, the microtubules of the distal end (bottom) do not spiral along their lengths but show "straight" or "wavy" configurations. Arrows point to microtubule termini.

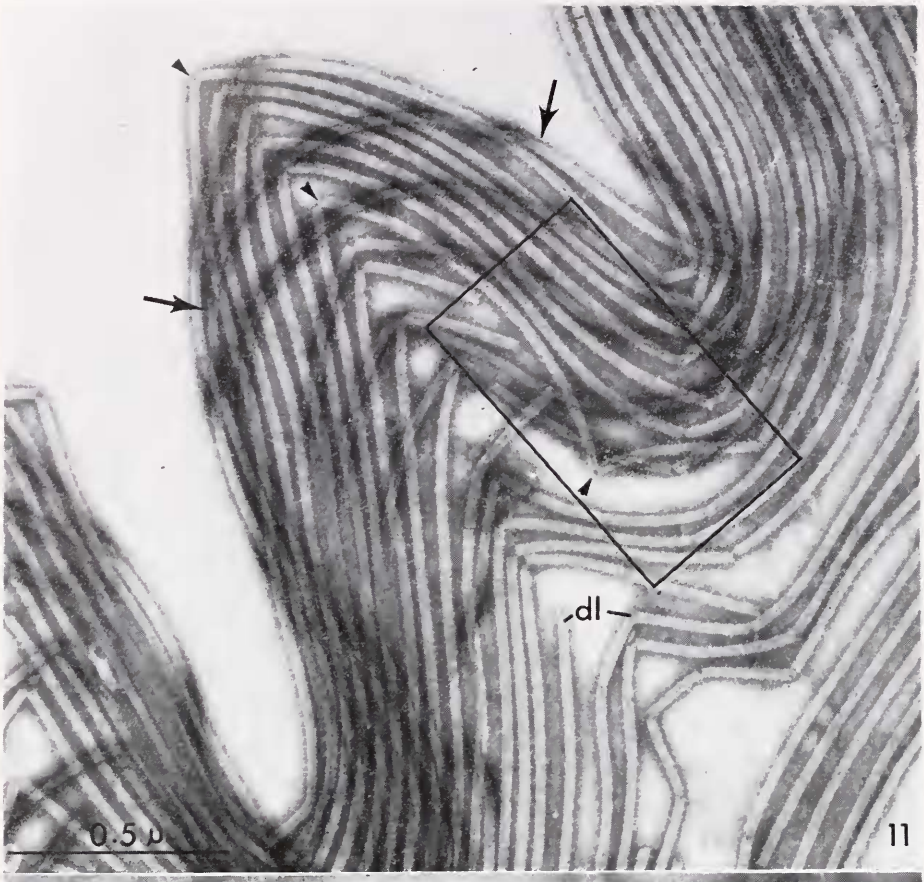
as invaginations from the surface of the spermatid and that secondary cytoplasmic extensions into the alveoli give rise to intra-alveolar cytoplasmic particles (Fig. 6). Mitochondria are included among the alveoli in the sparse cytoplasm of the spermatozoan mid-region. The alveoli do not extend into the ends of the seprmatozöon. In the proximal region of the gamete, membranous whorls, endoplasmic reticulum and many mitochondria are found within coarsely granular cytoplasm (Fig. 5).

In overall appearance the nucleus is electron-dense and therefore stands out in marked contrast to the cortical and alveolar regions of the spermatozoön (Figs. 4, 5 and 6). The nucleus is irregular in cross-sectional outline and is bounded by an envelope approximately 260 Å in thickness (Figs. 4 and 5). The nuclear contents are not uniformly electron-dense. In any section of the nucleus, electron-lucent and electron-opaque areas are observed in an apparently random pattern (Figs. 3, 4 and 5). Within electron-lucent areas, in transverse sections especially, fibers are seen (Fig. 4) ; these fibers are predominantly longitudinal in orientation (*cf.* Fig. 6). A similar fibrous component can be discerned within electron-opaque regions of the sperm nucleus. The fibers vary in diameter from 80–120 Å and are separated from one another by 110 to 130 Å (average 123 Å). A granular dark material provides the background for electron-opaque regions. The nucleus of a spermatid (Fig. 6) is not as electron-opaque as the nucleus of mature sperm (*cf.* Figs. 4 and 5). A longitudinal array of dark fibers is evident in dark and light areas of the condensing sperm nucleus. A few fibers are cut transversely in Figure 6. What nuclear components (as here defined by morphologic and electron-density criteria) constitute sperm chromatin in this form is not clear.

Discussion

In the classical sense, the spermatozoön of *Hydrolimax* cannot be said to have a head, neck, middle piece or tail (*cf.* Wilson, 1925). No structure resembling or functioning as an acrosome has been observed, nor have flagella or flagella-like bodies been observed in the mature spermatozoön or at any time/stage of spermatogenesis (Newton, unpublished data). Spermatozoa removed from the body of *Hydrolimax* into pond water exhibit no motility.

Jensen (1883), Böhmig (1891) and von Graff (1908, 1911) described living mature spermatozoa of some marine plagiostomid species. Except for spiraling of the sperm-cell body, reported by Jensen (1883) for *Plagiostomum vittatum* and by Böhmig (1891) for *P. girardi* and *P. reticulatum,* the spermatozoa of the marine species are morphologically similar to the spermatozoön of *Hydrolimax.* Böhmig (1891) observed that the central core (*Centralfaden* = sperm nucleus) of plagiostomid spermatozoa in histological preparations is composed of a proximal darkly-staining region and a distal lightly-staining region after hematoxylin and carmine stains. The proximal and distal regions were, according to Böhmig, composed of chromatic and achromatic nuclear substances. Jensen (1883) did not report regional variation in staining properties in the sperm nucleus of *P. vittatum;* none has been observed in spermatozoa of *Hydrolimax* in histological preparations. Böhmig (1891) and von Graff (1908, 1911) describe and figure spiral nuclei in spermatozoa in histological preparations of some marine plagiostomids. Spiral nuclei were not reported by Jensen (1883), and none was observed in the present study.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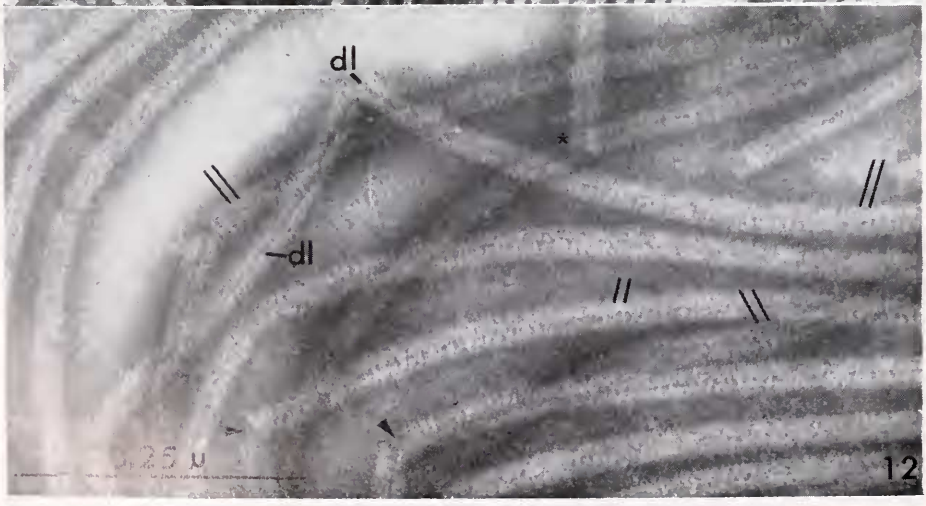
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Christensen (1961; p. 416) briefly described the mature spermatozoön of *Plagiostomum morgani:* in general morphology the spermatozoön of *P. morgani* is like that of *H. grisea,* ". . . spindle-shaped, tapering at each end into slender processes which are respectively 40 μ and 10 μ." The short and long processes may correspond to the proximal and distal ends, respectively, of the spermatozoön of *Hydrolimax.* The nucleus of the spermatozoön of *P. morgani* is fusiform and contains a "dense core" (electron-dense?) which protrudes from the end of the nucleus at the shorter end of the spermatozoön. No such dense nuclear core, nor protrusion, is seen in the nucleus of the spermatozoön of *H. grisea.*

After extensive molecular reorganization and condensation, the nuclear contents of the spermatozoa of most animals becomes uniformly electron-dense (André, 1963; Walker and Macgregor, 1968; Chevallier, 1970; Fawcett, Anderson and Phillips, 1971; Henley, 1973). There are exceptions, however, of which the nuclear contents of the spermatozoöon of *Hydrolimax* is another example. Von Bonsdorff and Telkka (1965; their Fig. 3) show the sperm nucleus of *Diphyllobothrium latum* to be electron-lucent, with scattered, finely-fibrillar, electron-dense material. Henley (1974) described the sperm nucleus of the polyclads *Stylochus* and *Notoplana* as diffuse, showing a lamellar array of electron-opaque fibers in an electron-lucent background (*cf.* Henley, 1974; Fig. 19). The nuclei of the spermatozoa of the acoels *Anaperus* and *Polychoerus* are "diffuse," without lamellar arrays of fibers. In *Polychoerus,* the spermatozoa have an electron-dense nuclear periphery (Henley, 1974; Fig. 21). Silveira and Porter (1964) were able to resolve the nuclear contents of the spermatozoa of *Dugesia tigrina, Bdelloura candida* and *B. propinqua* into two components: (1) a dense component constructed of lamellar or leaf-like subunits 60 Å thick, and (2) a less dense, finer-textured component. On the basis of staining affinity for uranyl acetate, Silveira and Porter (1964) concluded the dense component to be "chromatinic" and the lucent component to represent residual protein. In transverse section ". . . the chromatinic material comprises frequently a 'cruciform' figure, with four less dense areas representing the protein components disposed peripherally in the four quadrants of the cross . . ." (Silveira and Porter, 1964; p. 246). In a subsequent study of the nucleus of the spermatozoön of *D. tigrina,* Silveira (1970) characterized the proteinic (electron-lucent) nuclear component as a basic protein which she tentatively interpreted as being a homologue of an acrosome.

The "pellicle" (= cortex; *cf.* Christensen, 1961; p. 416) of the spermatozoön of *P. morgani* contains ". . . parallel fibrils or tubules . . . which pursue a spiral course up the body of the sperm." The cytoplasm of the mid-region of the spermatozoön of *P. morgani* ". . . contains whorls of flattened cisternae, resembling endoplasmic reticulum (without ribosomes), which extend out to insert along every other or every few pellicular fibrils" (Christensen, 1961; p. 416). Membranous whorls are observed occasionally in the proximal portion of the spermatozoön of *H. grisea,* but they do not appear to insert along the cortical singlet microtubules.

FIGURE 11. Singlet microtubules showing curves (large arrows) and breaks (small arrows). A dark line, probably PTA deposits, extends down the singlet centers. Outlined area is shown at higher magnification in Figure 12; dl represents dark line.

FIGURE 12. Pitch and spacing of subunits (parallel lines) suggest a helical substructure to singlets. A linear (protofilamentous) array is indicated at asterisk. Arrows indicate breaks; dl represents dark line.

Alveoli characterize the cytoplasm of the mid-region of the spermatozoön of *Hydrolimax*. On the presence or absence of centrioles in the spermatozoön of *P. morgani*, Christensen (1961) makes no comment. No centriole has been observed in any section of spermatozoa of *H. grisea* studied by electron microscopy. Likewise, centrioles have not been found in spermatozoa studied by light microscopy, in squashes, smears or sectioned and stained material.

Christensen (1961) found no evidence of flagella or of an undulating membrane in the spermatozoön of *P. morgani*. Hendelberg (1969) did not find flagella in the spermatozoa of other plagiostomids, *P. vittatum*, *P. cinctum*, *Vorticeros auriculatum* and *Acomostomum dioicum*, studied by phase microscopy.

Flagella and axonemes are absent from spermatozoa of a number of other turbellarian forms. The spermatozoa of *Macrostomum rubrocinctum*, studied by phase optics only, are aflagellate (Hendelberg, 1969). In a freshwater species of *Macrostomum*, collected near Chapel Hill, North Carolina, the spermatozoa, studied by phase-contrast and electron microscopy, are non-axonemal (Thomas and Henley, 1971). In some rhabdocoels, *Provortex balticus*, *Graffila buccinicola*, *Syndesmis echinorum* and *Proxenetes* sp., the spermatozoa are aflagellate as Hendelberg (1969) concluded from phase-contrast microscopy. Spermatozoa with no free flagella may have axonemes incorporated into the cytoplasm along the length of the body.

Mesostoma georgianum (Henley, Costello, Thomas and Newton, 1969), *Dugesia tigrina*, *Bdelloura candida* and *B. propinqua* (Silveira and Porter, 1964), and *Microdalyellia* sp. (Henley, 1974) have biflagellated spermatozoa, the bodies of which undulate independently of the two flagella. Spermatozoa of *Stylochus zebra* have no free flagella; they do have lateral cytoplasmic axonemes (Thomas, 1970). Cortical singlet microtubules are longitudinally aligned along the bodies of all these spermatozoa. Hendelberg (1970) observed, however, that the undulations of the body of the biflagellated spermatozoa of some polyclads cease when flagella are removed by mechanical means.

Mattei (1970) and Mattei, Mattei, Reizer and Chevalier (1972) reported aflagellate spermatozoa from six species of teleosts from Africa. In only one of the species, *Gymnarchus niloticus*, were cortical singlet microtubules observed in the spermatozoa; and in *G. niloticus* the spermatozoa exhibited a kind of ameboid motion. Mattei *et al.* (1972) were not able to observe living spermatozoa of the other teleosts.

Axonemes of the common "9 + 2" pattern of microtubules are conspicuously absent from the spermatozoa of coccid insects (Moses, 1970; Robison, 1972). Longitudinally-oriented singlet microtubules, concentric or spiral in transverse array, are the motile element of coccid spermatozoa (Moses, 1970; Robison, 1972). In the aflagellate spermatozoön of the dipteran, *Rhynchosciara* sp., a "flagellar complex" of over 350 doublet microtubules constitutes the motile apparatus (Shay, 1972).

The spermatozoa of *Macrostomum* sp. (Thomas and Henley, 1971), of *M. retortum* and *Paramacrostomum quiesztori* (Bedini and Papi, 1970), with cortical singlets as their only microtubular elements, are the only verified non-axonemal sperm of Turbellaria known to be motile. There exists a tacit implication of motility in the spermatozoa of the other turbellarians discussed above. Hendelberg

(1970) notes that the aflagellate spermatozoa of some Turbellaria are capable of active motility, especially the spermatozoa of the plagiostomids. There is only one description in the literature, of which the present author is aware, of movement by the aflagellate spermatozoön of a plagiostomid; Jensen (1883; p. 22) made one observation of "legers mouvements d'oscillations de la queue" of a spermatozoön of *Plagiostomum vittatum*. In the absence of more direct observations, it is assumed that, since the sperm are injected into the parenchyma of copulating adults, the spermatozoa actively migrate to the vicinity of eggs. The spermatozoa may require some sort of capacitation or other activation within the maternal parenchyma to become motile. The importance of the substrate through which plagiostomid spermatozoa travel under normal conditions to effect fertilization cannot be overlooked. The interaction between spermatozoa and parenchymal cells of adult turbellarians remains to be investigated. Christensen (1961, p. 416) assumed the spermatozoa of *P. morgani* to ". . . proceed by some sort of wriggling motion." He proposed the cortex to be ". . . the basis of motility, perhaps through differential contractions of the pellicular fibrils over the surface of the sperm" (Christensen, 1961; p. 416). Possibly the cortical singlets, alone or in concert with some cortical factor or substance, are responsible for motility in aflagellate, non-axonemal turbellarian spermatozoa.

How the sperm move is unknown. If cortical singlet microtubules, reported in spermatozoa of *Plagiostomum* and *Hydrolimax*, are responsible for movement, then the question is: how? In the mid-region of the sperm of *Hydrolimax*, the microtubules are widely separated. In the compressed distal portion of the sperm microtubules are close. No evidence of bridging between microtubules has been observed in *Hydrolimax*. The "fibrils or tubules" of the spermatozoön of *Plagiostomum* are spaced 700 Å apart (Christensen, 1961). The distances over which microtubules are capable of interacting are unknown. There is a possibility that singlet microtubules reported here may serve *solely* as cytoskeletal elements.

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Summary

1. The structure of the aflagellate, non-axonemal spermatozoön of *H. grisea* was studied by phase contrast optics and electron microscopy.

2. The gamete is fusiform with a centrally-located, vermiform nucleus surrounded by cytoplasmic alveoli.

3. The alveoli and intra-alveolar inclusions are membrane-bound vesicles and membrane-bound particles, respectively. The latter resemble sperm cytoplasm and are believed to be cytoplasmic extensions into alveoli.

4. The nucleus has an irregular pattern of electron-opaque and electron-lucent areas. In predominantly longitudinal orientation are dark fibers, *ca.* 95 Å separated by 123 Å, coursing through the opaque and lucent areas of the nucleus.

5. Cortical singlet microtubules are the only microtubular elements in the spermatozoön of *Hydrolimax*. The array and discernible substructure of the singlets are described from sectioned and PTA-macerated material. The *possible* role of the singlet microtubules in sperm motility in *H. grisea* is discussed.

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